## REMARKS

## Claim Amendments

In this response, claim 1 has been amended and claim 10 has been added. Support for the amendments to claim 1 can be found, for example, on page 9, lines 14-18. Support for claim 10 can be found, for example, on page 4, lines 13-15, and Figure 1. No new matter has been added by these amendments and all amendments have been made without prejudice.

## Claim Rejections - §102

The nanobubbles of the claimed invention are generated in a manner to provide a structure such that "the nanobubbles remain in the solution for at least one month", as a result of abruptly reducing in size microbubbles contained in an aqueous solution containing electrolytes of "ferrous ions, manganese ions, calcium ions, sodium ions, magnesium ions or any other mineral ion" by applying physical irritation thereto, as described 1 of the present invention. In addition, the present invention has structures and effects described in claims 2, 3, 4 and 5 of the present invention in the step of abruptly reducing in size the microbubbles. Particularly, as described claim 3 of the present invention, mineral ion or other ions is a shell around the nanobubbles, as a result, the effect of stabilizing the nanobubble for as much as one month or more months is caused. Further, regarding claim 10, the nanobubbles have a "medium particle diameter of about 140 nm with a standard deviation of about 30 nm"

When the claimed invention of the claims is compared with Wheatley et al., the fact that the nanobubbles are stabilizing for at least one month is neither taught or suggested by Wheatley. In addition, neither the structure described in the claims 3-5 of the present invention nor the effect brought by them also is taught or suggested by Wheatley.

Therefore, the claims 1 and 3-5 of the present invention are patently distict from Wheatley. Therefore, the Applicants respectfully request the withdrawal of the anticipation rejections.

## Claim Rejections - §103

The present invention is based on the Applicants study of microbubbles lasting for 20 years or

more. From this study, the Applicants understand the following facts:

- a) the microbubble is a bubble having a diameter of  $10-50~\mu m$  and disappears in water (this is because an internal gas is effectively dissolved);
- b) the microbubble takes electrical charge caused by the adsorbed ions at the gas-water interface of the tiny bubble, and the adsorbed ions are H<sup>+</sup> and OH<sup>-</sup> generated by the electrolytic dissociation of water:
- c) the charge increases by the shrinking process of the microbubble under water;
- d) the increase ratio of the bubble charge is proportional to the shrinking speed of the microbubble; and
- e) the charged surface of the microbubble attracts the counter ions including the electrolyte ions such as  $Na^+$  and  $Mn^{2+}$ , and these ions suppress the dissolution of the interior gas of the shrinking microbubble.

In researching the present invention, the Applicants discovered:

- f) the generated nanobubble from the shrinking microbubble by accelerating the shrinking speed using the physical stimuli is covered by condensed electrolyte ions;
- g) when the bubble has been shrunk to about 140nm (standard deviation of about 30 nm) in diameter, the bubble stabilizes for long period under water with longevity for one month or more; and
- h) the water containing these nanobubbles has specific useful properties, such as the activation (rejuvenation) of fish.

The point of the present invention based on the knowledge of the above a)-h) is to give the microbubble the physical stimulation and to reduce compulsorily after preliminarily making the microbubble having a diameter of  $10-50~\mu m$ . In common sense, it has been thought that the microbubble disappears at once when this is done. However it is possible for the nanobubble to exist in making after one month according to the present invention (refer to claims 1 and the EXAMPLE 1 in the present specification).

The present invention has disclosed the generating method of the long lifetime nanobubble.

which is strongly electrically charged and has specific useful properties not found in the prior art nano/micro bubbles.

All of the disclosures in five cited references (WO03/0227356, JP60-122337, Bunkin et al., McGrath et al., and Aquarius), disclose that minute bubbles are generated immediately, with the implication that the bubbles dissolve or burst after a short time. The prejudice at the time of the prior art was that the nanobubble disappears quickly because an internal gas is dissolved though the bubble. These prior art bubbles have a half life of a few minutes to several hours depending on the method used to make the bubble. Moreover, because pressure in the bubble is high, the amount of dissolution of the gas in water rises when the prior art microbubble is generated. The disclosure in other cited prior art references also show that the density of the dissolution gas is very high. Though this shows that the made bubble is extremely small, this also shows that the bubble structure is not stable and the bubbles are breaking quickly.

WO03/0227356 disclosed how to generate the highly concentrated ozone water by a water discharge with the existence of the gas phase as the form of fine bubble in the discharge area. Oxygen gas is changed to ozone gas by the discharge. Since after the discharge the ozone remover removes the ozone gas, the fine bubble (gas phase) does not exist in the water. So the invention of WO03/0227356 does not disclose how to generate the long lifetime (one month or more) nanobubbles.

JP60-122337 disclosed how to generate the high ozone concentration by using turbulent flow. It means that the turbulent flow is used to <u>collapse</u> the tiny bubbles, not to prolong the lifetime of the bubble. This teaches away from the stability structure feature of the claimed invention.

Bunkin et al. disclosed that bubstons could be work as bubble nuclei for the optical cavitations.

Bubble nuclei easily expand to the macroscopic bubbles according to the change in environmental condition. These bubbles cannot exist for one month or more.

McGrath et al disclosed that oxygen nanobubbles as small as 20-30 nm can supply the oxygen

concentration significantly higher than 250 ppm to tissues, and they form irregular networks that nearly completely cover hydrophobic surfaces. Since the high oxygen concentration is derived from the nanobubble, these nanobubbles have short lifetime. The nanobubbles are distinct from the present invention.

On the other hand, nanobubbles of the claimed invention are stable for one month or more. The fact that the bubbles remaining stable for a long time means that they don't become the supply medium of the gas in water. Therefore, the density of the dissolution gas is <u>not</u> supersaturated in the present invention.

To reiterate, the point of the present invention is i) the nanobubble is obtained by giving physical stimulation to a ion solution containing microbubbles that is reduced and stabilized and ii) a bubble with stability for one month or more though the nanobubble is a size of nano level (refer to claim 1 and the EXAMPLE 1 in the present specification). The present invention (claims 1-5 and 9 and 10 of the present invention) is not found by combining the five cited references.

Therefore, the Applicants respectfully request that the obviousness rejections be withdrawn.

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October 6, 2010
(Date of Transmission)

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